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2. Cancer Research, 2002, 62(15):4263-4272

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ACCESSION NUMBER: 2002:951264 CAPLUS
DOCUMENT NUMBER: 139:272943
TITLE: Direct electrophilic radiofluorination of a cyclic RGD peptide for in vivo .alpha.v.beta.3 integrin related tumor imaging
AUTHOR(S): Ogawa, Mikako; Hatano, Kentaro; Oishi, Shinya; Kawasumi, Yasuhiro; Fujii, Nobutaka; Kawaguchi, Michiya; Doi, Ryuichiro; Imamura, Masayuki; Yamamoto, Mikio; Ajito, Keiichi; Mukai, Takahiro; Saji, Hideo; Ito, Kengo
CORPORATE SOURCE: Department of Biofunctional Research, National Institute for Longevity Sciences, Gengo, Morioka-cho, Obu, 474-8522, Japan
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AUTHOR(S): Kessler, Horst; Kantlehner, Martin; Gibson, Christoph; Haubner, Roland; Finsinger, Dirk; Dechantsreiter, Michael; Planker, Eckart; Wermuth, Jochen; Schmitt, Jorg S.; Meyer, Jorg; Schaffner, Patricia; Holzemann, Gunter; Wiesner, Matthias; Goodman, Simon L.; Hahn, Diane; Jonczyk, Alfred; Wester, Hans J.; Schwaiger, Markus
CORPORATE SOURCE: Institut fur Organische Chemie und Biochemie, Technische Universitat Munchen, Garching, 85747, Germany
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Recent progress in the field of α_v-integrin antagonists

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Introduction

Integrins - a class of heterodimeric, transmembrane glycoprotein receptors - play an important role in many physiological processes. The development of highly active and selective integrin antagonists is a promising approach for the treatment of various diseases. Cyclization and "spatial screening" yielded cyclo(RGDfV) [1,2] as highly active and selective $\alpha_v\beta_3$-integrin antagonist. Extensive peptidomimetic studies finally culminated in cyclo[RGDf-N(Mc)V] [3,4] which binds in the subnanomolar range and is selected for clinical phase I/II as as antiangiogenetic tumor drug (EMD 121974). Their NMR derived structures are shown in Fig. 1.

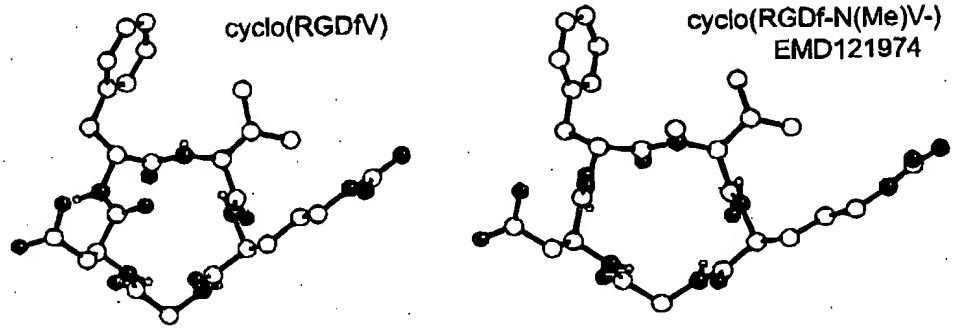


Fig. 1. NMR-derived solution structure of cyclo(RGDfV) and its N-methylated analog cyclo[RGDf-N(Me)V]. The N(Me) group imparts steric regulation via the peptide backbone resulting in a widened arrangement of the pharmacophoric RGD sequence. The structures show one of the side-chain conformations which interconvert fast in solution.

We report here about improving the activity, selectivity and bioavailability of these antagonists and the development of non-peptidic analogues via combinatorial techniques. Furthermore we functionalize our antagonists for special applications, e. g. surface coating or radionuclide medicine.

Results and Discussion

We found that X in cyclo(RGDfX) can be replaced by other amino acids without a remarkable change in activity and selectivity [5,6]. Replacement by Lys or Glu introduce useful functionalities for any derivatizations of the cyclopeptides.

For application in radionuclid diagnostic we synthesized the radiolabeled peptide with ^{125}I-D-Tyr instead of D-Phe: cyclo[RGD(^{125}I)yV]. After modifying the peptide by conjugation of a sugar amino acid (SAA) to the Lys side chain (X = K) the biodistribution and tumor accumulation of the glycosylated peptide cyclo(RGD(^{125}I)y[SAA]K) exhibited drastically improved biokinetics [7] compared with the non-glycosylated compound.

For biofunctionalization of inert surfaces we have coupled our highly active and $\alpha_v\beta_3$- and $\alpha_v\beta_5$-selective peptide cyclo(RGDfK) over the lysine side-chain to various linker-molecules containing acrylic acid as anchor functionality [8]. These peptides can be covalently linked to polymethylmethacrylate-(PMMA)-surfaces (Fig. 1). In contrast to untreated surfaces the coated surfaces bind murine osteoblasts as well as human osteoblasts very effectively *in vitro* if a critical minimum distance of 3.5 nm between surface and the constrained RGD-sequence is ensured (Fig. 2).

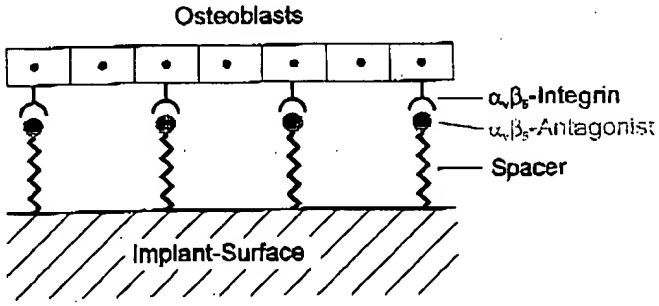
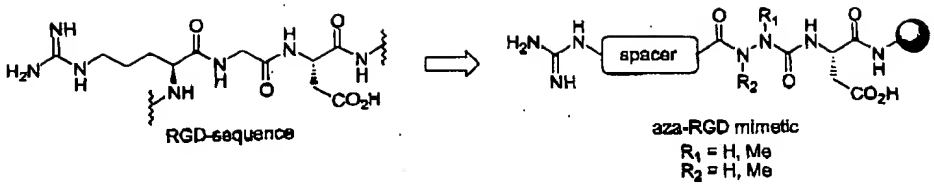


Fig. 2: Schematic function of RGD-coated surfaces.

Promising cell adhesion rates and strong cell attachments were observed, and surface bound cells started to proliferate. These findings may be helpful for the development of a new generation of bone implants as the peptides can provide a strong mechanical contact between implant surface and the surrounding healthy and new forming tissue.

Substitution within the RGD sequence in the lead peptide cyclo(RGDfV) mostly resulted in a loss of activity and selectivity. However, a remarkable difference was found with azaGly instead of Gly. This cyclic peptide exhibited full activity and according to NMR and DG studies showed a twisted NN bond [9,10]. This observation stimulated us to search for linear azaGly analogues and we found that we can modulate activity and selectivity in linear diacylhydrazines as well [11]. We found suitable reaction conditions to prepare activated Fmoc-protected azaglycine, azasarcosine and azaalanine in high yields, by treatment of the corresponding Fmoc-hydrazines with a commercially available solution of phosgene in toluene. To check the feasibility of preparing azapeptides and azapeptoids on a solid support, we carried out a systematic study of the coupling conditions and targeted the preparation of some RGD-mimetics, all of which contain azabuilding blocks instead of glycine.



The synthesized aza-RGD-mimetics exhibit varying activity and selectivity on the integrin receptors $\alpha_v\beta_3$ or $\alpha_{IIb}\beta_3$ depending on the substitution pattern of the azabuilding block. The results offer a potential application of azapeptides and azapeptoides as selectivity and activity inducing templates in pseudo biooligomers. We want to emphasize that our strategy afforded completely deprotected Fmoc-aminoethyl-photolinker [12] TentaGel-bound RGD-mimetics, which meet all requirements of the one-bead-one-compound concept [13] and allowed biological on-bead screening and subsequent chemical characterization via MS^n, due to orthogonal anchoring. For that purpose we have developed an on-bead assay for biological evaluation of aza-RGD-libraries.

Acknowledgments

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